Advanced Hemostatic Dressing Development Program: Animal Model Selection Criteria and Results of a Study of Nine Hemostatic Dressings in a Model of Severe Large Venous Hemorrhage and Hepatic Injury in Swine

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Background: **An advanced hemostatic dressing is needed to augment current methods for the control of life-threatening hemorrhage. A systematic approach to the study of dressings is described. We studied the effects of nine hemostatic dressings on blood loss using a model of severe venous hemorrhage and hepatic injury in swine.**

Methods: **Swine were treated using one of nine hemostatic dressings. Dressings used the following primary active ingredients: microfibrillar collagen, oxidized cellulose, thrombin, fibrinogen, propyl gallate, aluminum sulfate, and fully acetylated poly-*N*-acetyl glucosamine. Standardized liver injuries were induced, dressings were applied, and resuscitation was initiated. Blood loss, hemostasis, and 60-minute survival were quantified.**

Results: **The American Red Cross hemostatic dressing (fibrinogen and thrombin) reduced ($p < 0.01$) posttreatment blood loss (366 mL; 95% confidence interval, 175–762 mL) and increased ($p < 0.05$) the percentage of animals in which hemostasis was attained (73%), compared with gauze controls (2,973 mL; 95% confidence interval, 1,414–6,102 mL and 0%, respectively). No other dressing was effective. The number of vessels lacerated was positively related to pretreatment blood loss and negatively related to hemostasis.**

Conclusion: **The hemorrhage model allowed differentiation among topical hemostatic agents for severe hemorrhage. The American Red Cross hemostatic dressing was effective and warrants further development.**

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The development of improved methods for hemorrhage control is a major emphasis within the U.S. Army Combat Casualty Care Research Program. In combat, casualties may not be evacuated to advanced care for hours or days, depending on the tactical situation. Hemorrhage before evacuation accounts for 49% of overall battle deaths, whereas hemorrhage after evacuation accounts for just 1% of overall battle deaths from wounds.[1] There is a population of combat casualties that are potentially salvageable with improved methods, drugs, or devices for emergency treatment.[2,3] Among those that died from combat wounds in Korea, Vietnam, and Somalia, 7% to 14% died from extremity hemorrhage.[4,5] There is great potential for an advanced hemostatic dressing (HD) to reduce overall combat deaths from wounds. In this article, we present our approach to animal model selection and results of a study of nine potential HDs for treatment of severe venous hemorrhage.

An advanced HD development integrated product team was assembled to identify requirements, select animal models, and plan the research and development program. Although diffuse bleeding in coagulopathic patients can be a lethal problem,[6] the more important problem is bleeding from larger structures.[7,8] These deaths occur rapidly.[1,9–12] The requirement for an HD was the rapid control of life-threatening hemorrhage before evacuation. The primary HD patient was identified as a young, healthy service member with a major, life-threatening, actively bleeding, vascular wound that is accessible to the medic. This patient will have a normal coagulation system at the time of wounding. Primary HD use will be medic or buddy aid.

It was important that model selection criteria ensure relevance to hemorrhage severity and coagulation status of the target patient, and no artificial bias in favor of or against

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any specific product or product mode of action. A range of animal models of uncontrolled hemorrhage was reviewed and considered. The principal differences among models related to the method of hemorrhage induction, source of hemorrhage, and model severity.

Hemorrhage induction methods include variations of incision, penetration, transection, or crushing. Skin[13,14] or mucosa can be incised.[14] Vessels may be incised[15–17] or torn,[18] or a portion of the vessel wall may be removed with a punch.[19] Organs may be incised[20–22] or portions of organs may be excised[20,23–27] or removed by a punch.[28] Another approach is to incise and strip capsules from sections of organs.[20,21] Methods involving penetration or puncture of a vessel may penetrate one[29,30] or both sides.[31] Organs can be sharply penetrated[32–36] or stabbed.[37] Transection may involve transection of an individual vessel,[38] a portion of the tail,[39] or the cuticle.[13] Crush injuries often involve solid organs.[28] Models of blunt abdominal trauma[40] and extremity gunshot[41] have been reported. These models involve complex injuries that include combinations of the types of defects discussed above. Quantitation of hemorrhage was also a factor. Most models directly measure blood loss,[15,18,23,25–27,32–35,40–42] whereas others use indirect methods.[13,30] Bleeding time may be determined by direct observation and continuous timing[14,25] or expressed as the required number of dressing compressions or applications.[20–22,29,42]

Other important considerations pertain to the size of the defect and the severity of the hemorrhage. The geometric area of the vascular defect and the pressure gradient across the vessel wall are the main determinants of blood loss.[43] Vessel diameter is vessel and species specific. The tension within the vessel wall is related to the pressure pushing against the vessel wall and the radius of the vessel, according to the law of Laplace: Tension = Pressure × Radius.[44] For an HD to seal a damaged vessel, the clot or other seal formed must hold under the tension characteristic of the given vessel wall. The physiologic severity of the hemorrhagic insult can be determined on the basis of lethality in the absence of treatment. Severity can also be assessed from the standpoint of difficulty to control bleeding. Flowing hemorrhage from a large defect in a large vessel may be considered severe, whereas capillary bleeding that is temporarily controlled using vascular clamping before application of the experimental hemostat may be considered mild. The status of the hemostatic mechanism differs among animal models. Hemorrhage models often use anticoagulants or other methods to achieve adequate hemorrhage or investigate coagulopathic states.[13,14,22,23,25,28,29,33,35]

Models of arterial, large venous, and diffuse bleeding were required. To address venous bleeding, we selected a model of severe venous hemorrhage and liver injury in swine.[32,34,36] Although this model includes large incisions through liver parenchyma, the major hemorrhage is vascular. Intra-abdominal hemorrhage is not an exact match with the accessible hemorrhage of the primary patient. This limitation was seriously considered in model selection. The model was selected on the basis of several desirable characteristics, including large-diameter veins, ability to apply HD in the presence of free-flowing hemorrhage, ease of blood loss quantitation, ability to determine time to hemostasis, ease of instrumentation, potential for lethality, no requirement for anticoagulation, and reproducibility.

As a part of the program to identify an advanced HD for military use, a request for proposals for HDs was published in February 1999. Nine HDs were submitted, including both commercially produced and experimental HDs. We examined the effects of these HDs on blood loss, hemostasis, and short-term survival. This study was completed in early 2000.

MATERIALS AND METHODS

Animals

Crossbred commercial swine were used in this study. Animals were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. This study was approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas. Animals received humane care in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 86-23, revised 1996).

Hemostatic Dressings

Because nine different HDs were included in this study, it would have been impractical to include a separate placebo control for each product. Therefore, a standard 10.2 × 10.2-cm gauze sponge was selected as a control (Nu Gauze general-use sponges, Johnson & Johnson Medical, Inc., Arlington, TX).

Alltracel Laboratories (Spol. S R. O., Tišnov, Czech Republic) submitted the Hemostatic Dressing Pad. This dressing was a multilayer hemostatic and absorptive dressing, involving a hemostatic contact layer and two superabsorbent layers. The active substance of the contact layer was a neutral microfibrillar quasi-nonwoven form of the company's proprietary microdispersed oxidized cellulose.

The American Red Cross (Rockville, MD) submitted a dry fibrin dressing. This dressing consisted of 15 mg/cm^2 of human fibrinogen, 37.5 U/cm^2 of purified human thrombin, 3.25 U/cm^2 factor XIII, and 40 mmol/L $CaCl_2$/cm^2 freeze-dried onto an absorbable polygalactin mesh backing measuring 10.2 × 10.2 cm (Vicryl, Ethicon, Inc., Somerville, NJ).

Analytical Control Systems, Inc. (Fishers, IN), submitted gauze sponges (10.2 × 10.2 cm) that were soaked in Hemostatin and lyophilized. The active component of Hemostatin is propyl gallate.

Clarion Pharmaceuticals, Inc. (Westlake, OH), submitted the Hemarrest dressing. This dressing was a thin, sheet-like pad with a mixture of epsilon aminocaproic acid and thrombin on the active side.

Davol, Inc. (Woburn, MA), submitted the Avitene dressing. This dressing consisted of microfibrillar collagen prepared as a dry, sterile, fibrous, water-insoluble partial hydro-

chloric acid salt purified bovine corium collagen. It was prepared in a compacted nonwoven web form (7.0 × 7.0 cm).

Ethicon, Inc. (Somerville, NJ), submitted the Surgicel dressing. This consisted of a fibrillar material in the form of a sterile, absorbable, knitted fabric prepared by controlled oxidation of regenerated cellulose (5.1 × 10.2 cm).

Hemostace, LLC (New Orleans, LA), submitted Sorbastace Microcaps, which consisted of aluminum sulfate, USP, microcaps (150 μm) with a 6.0% ethyl cellulose coating. These were applied to standard (control) gauze sponges at the time of application.

Marine Polymer Technologies (Danvers, MA) submitted a fully acetylated poly-*N*-acetyl glucosamine dressing. The active component was formulated into a fully acetylated, lyophilized, sterile membrane measuring 10.2 × 10.2 cm.

Nycomed (Linz, Austria) submitted the TachoComb-S dressing. This dressing measured 4.8 × 9.5 cm and consisted of human fibrinogen and thrombin on a backing of equine collagen.

Experimental Procedure

Animals were placed in decreasing body weight order and assigned randomly to treatment in permutations of 10. Treatment groups included a gauze sponge control (GAU) and the dressings supplied by Alltracel Laboratories (ALL), American Red Cross (ARC), Analytical Control Systems (ACS), Clarion Pharmaceuticals (CLA), Davol (DAV), Ethicon (ETH), Hemostace (HEM), Marine Polymer Technologies (MPT), and Nycomed (NYC). Distribution of male and female pigs was equalized among treatment groups.

Surgical preparation consisted of the following. Animals were fasted 36 hours before the surgical procedure, with water allowed ad libitum. After premedication with glycopyrrolate and a combination of tiletamine HCl and zolazepam HCl (Telazol, Fort Dodge Laboratories, Fort Dodge, IA), anesthesia was induced by mask using 5% isoflurane. The swine were intubated, placed on a ventilator, and maintained with isoflurane. Carotid arterial and jugular venous catheters were placed surgically. Laparotomy was performed and splenectomy and urinary bladder catheter placement were completed. A rectal temperature between 37.0° and 39.0°C, arterial blood pH between 7.35 and 7.45, and 15 minutes of stable mean arterial pressures (MAP) were required before further experimental procedures. Blood pressure and heart rate were recorded at 10-second intervals throughout the study period using a continuous data collection system (Micro-Med, Louisville, KY). Baseline arterial blood samples were collected from each animal to confirm that each animal exhibited normal platelet count, prothrombin time, activated partial thromboplastin time, and plasma fibrinogen concentration.

Liver injuries were induced as previously reported.[32] The liver was retracted by manually elevating the left and right medial lobes to allow adequate exposure. Next, a specially designed clamp with two 4.5-cm sharpened tines configured in the form of an X was positioned with the center approximately 2 to 3 cm dorsal to the intersection of the left and right medial lobes, on the diaphragmatic surface of the liver. The base plate of the instrument was positioned beneath the quadrate lobe, on the visceral surface. The injury was induced by clamping the tines of the instrument through the parenchyma and underlying vessels of the two medial lobes so that the tines were seated in corresponding grooves in the base plate of the instrument. After the first penetration of the liver, the instrument was opened and the tines were withdrawn and repositioned to the animal's left such that the second application would overlap the first by 50%. After this repositioning, the liver was penetrated a second time. Documentation of the liver injury was achieved by excision and inspection of the liver at the conclusion of the experimental period. The injuries appeared as large stellate wounds with a small island of tissue in the center, and measured approximately 10 × 8 × 4 cm. Each injury completely penetrated the liver, and one or more of the left medial lobar vein, right medial lobar vein, and portal hepatic vein was lacerated.

Resuscitation was initiated 30 seconds postinjury with warm (38°C) lactated Ringer's solution in all animals. Resuscitation continued until baseline MAP was reached and was reinitiated if MAP decreased, throughout the 60-minute study period. Fluid was administered at 260 mL/min. Simultaneous with initiation of resuscitation, treatments were applied as follows. One dressing was applied to the surface of the quadrate lobe to cover the penetrating injury and two other dressings were applied to the injury from the diaphragmatic aspect. All HDs were used within 10 minutes of opening. Application of HDs was in accordance with manufacturers' recommendations, except that prewetting of the HD was not allowed and the compression sequence was standardized to the following. Compression was applied for 60 seconds in the dorsoventral direction. After 60 seconds, the injury was inspected to determine whether hemostasis was achieved. Next, the applicator's hands were repositioned and pressure was applied for 60 seconds in the lateromedial direction, and the observation for hemostasis was repeated. This sequence was repeated for a total of four 60-second compressions. If hemostasis was complete after any compression, no further compressions were performed. Hemostasis was defined as the absence of visually detectable bleeding from the injury site.

After completion of treatment application, the abdomen was closed and the animal was monitored for 60 minutes after injury or until death, whichever came first. Death before 60 minutes was defined as a heart rate of 0 for 1 minute. At 60 minutes, surviving animals were killed by an overdose of pentobarbital.

Immediately after induction of the injury, blood was continuously suctioned from the peritoneal cavity until the start of treatment application. The volume was determined and designated as pretreatment blood loss. At the end of the study period, each abdomen was opened and the liquid and clotted intraperitoneal blood was suctioned and measured. This was designated as posttreatment blood loss. In addition,

total resuscitation fluid use was recorded. Preinjury animal blood volume was estimated as previously reported.[34]

At the termination of the study, the liver was excised. The adherence strength of each dressing was then subjectively scored. The adherence strength scoring scale consisted of a range of scores from 1 through 5. A score of 1 indicated no adherence; 2 indicated slight adherence; 3 indicated adherence adequate to cause stretching of tissue in contact with the dressing without lifting the liver from the table; 4 indicated that dressing adherence was sufficient to partially lift the liver from the table; and 5 indicated that the dressing adherence was sufficient to completely lift the liver from the table, when the dressing was grasped and lifted with forceps. For analysis, the mean score from the three dressings within each animal was treated as a single value for adherence strength. Next, the number and identification of vessels lacerated was documented for each liver.

Animals were excluded from the study before injury induction according to the following exclusion criteria: hepatic abnormalities that may alter injury characteristics; inability to maintain a stable MAP between 50 and 90 mm Hg during the preinjury stabilization period; and starting laboratory values outside the following ranges: hematocrit, 23.3% to 42.1%; hemoglobin, 7.9 to 13.5 g/dL; platelets, $\geq$ 200,000/μL; prothrombin time, $\leq$ 14.0 seconds; and partial thromboplastin time, $\leq$ 25.0 seconds. In addition, animals were excluded from the study and all data eliminated if the injury did not completely penetrate the liver; one or more of the left medial lobar vein, the right medial lobar vein, or the portal hepatic vein were not lacerated; or a blood vessel located outside of the left medial lobe, the right medial lobe, or the quadrate lobe was lacerated.

Statistical Analysis

After six animals were entered into each treatment group, the data were examined nonstatistically to determine which HD treatment groups would not be included in the remainder of the study. The following preplanned criteria were applied: 0% survival or 0% hemostasis. Treatment groups that met one or both of these criteria (excluding the GAU group) were eliminated from further study. After completion of the study, the following procedures were used to analyze the data. The SAS statistical program was used for all analyses.[45] All continuous data were analyzed by analysis of variance using the GLM procedure of SAS. Body weight, estimated blood volume, number of vessels lacerated, baseline MAP, survival time, preinjury MAP, pretreatment blood loss, and hematologic data were analyzed using a model that accounted for the effects of treatment. Data are reported as mean $\pm$ SEM. Data were examined for heterogeneity of variance and nonnormality. These conditions were detected for posttreatment blood loss and fluid use data. Therefore, blood loss and fluid use data were log transformed before analysis. Blood loss and fluid use data were analyzed using a model that accounted for the effects of treatment and included the number of vessels lacerated as a covariate. These data are expressed as back-transformed means and 95% confidence intervals. The effects of number of vessels lacerated, attainment of hemostasis, and animal sex were also analyzed across treatment groups for selected variables. Blood pressure data collected over time were analyzed using a model that accounted for the effects of treatment, time, and treatment-by-time interaction. Distribution of female and male pigs, hemostasis, and survival data were analyzed by χ^2 or Fisher's exact test using the FREQ procedure of SAS. Data are reported as percentages. Adhesion strength scores were analyzed by Wilcoxon rank sum test using the NPAR1WAY procedure of SAS. Correlation was performed using the CORR procedure of SAS. Adhesion scores are reported as arithmetic means and standard errors of the mean. Two-sided tests were used for all comparisons. All comparisons among treatment groups were preplanned and were made between each HD treatment group and the GAU group using Dunnett's test where appropriate, or else a Bonferroni correction.

Table 1 Baseline and Preinjury Animal Characteristics

Variable	Value (Mean $\pm$ SEM)
Body weight (kg)	43.4 $\pm$ 0.4
Estimated blood volume (mL)	3,061 $\pm$ 21
Female/male (n/n)	46/43
Baseline MAP (mm Hg)	63 $\pm$ 1
Preinjury MAP (mm Hg)	52 $\pm$ 1
Hematocrit (%)	36.6 $\pm$ 0.4
Hemoglobin (g/dL)	12.7 $\pm$ 0.9
Platelets (1,000/μL)	580 $\pm$ 18
PT (s)	11.6 $\pm$ 0.1
aPTT (s)	16.5 $\pm$ 1.0

PT, prothrombin time; aPTT, activated partial thromboplastin time.

RESULTS

There were no differences among treatment groups in animal body weight, estimated blood volume, distribution of animal sexes, baseline MAP, preinjury MAP, number of major vessels lacerated within the liver injury, pretreatment blood loss, or baseline hematologic values, when analyzed after either n = 6 or n = 11. Data for all animals are shown in Tables 1 and 2. There were no effects of animal sex on any variable measured.

After six animals were completed in each treatment group, four treatment groups were eliminated from further study, on the basis of the criteria listed above. The CLA,

Table 2 Injury Characteristics

Variable	Value (Mean $\pm$ SEM)
No. of vessels lacerated	2.3 $\pm$ 0.1
Pretreatment blood loss (mL)	317 $\pm$ 18
Pretreatment blood loss (mL/kg body weight)	7.3 $\pm$ 0.4

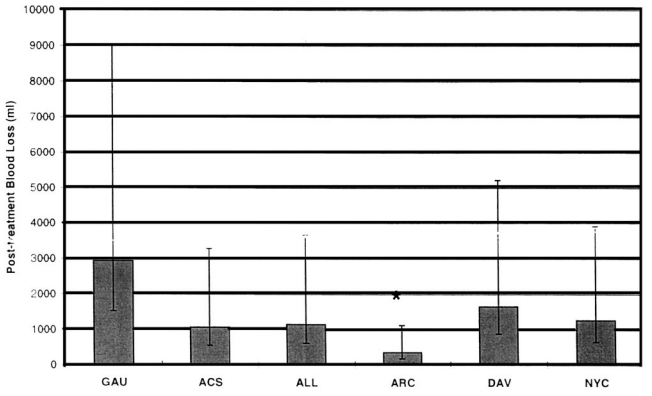

Fig. 1. *Effect of hemostatic dressing type on posttreatment blood loss. *Different from gauze control (p < 0.01). Means and 95% confidence intervals are shown.*

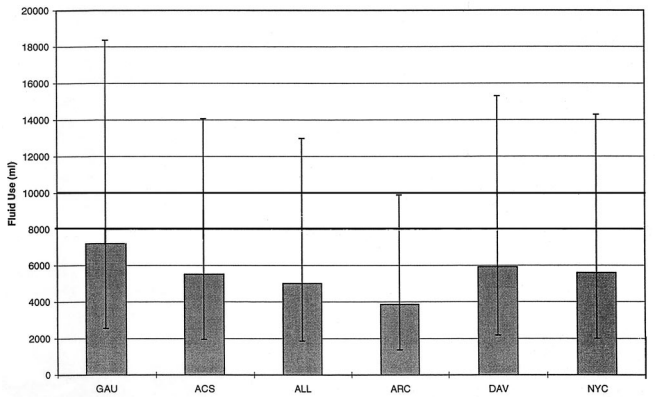

Fig. 3. *Effect of hemostatic dressing type on resuscitation fluid volume. Means and 95% confidence intervals are shown.*

ETH, HEM, and MPT groups were dropped. The group values for hemostasis, survival, and posttreatment blood loss, respectively, were as follows: CLA, 0 of 6, 0 of 6, and 5,616 ± 287 mL; ETH, 0 of 6, 4 of 6, and 2,513 ± 1,144 mL; HEM, 0 of 6, 0 of 6, and 4,546 ± 493 mL; and MPT, 0 of 6, 2 of 6, and 4,556 ± 836 mL. At the time that these groups were dropped, no differences among treatment groups were noted for any animal or injury characteristic. No statistical comparisons of outcome variables were made between these groups and any other group.

Analysis of data after completion of the full study revealed that posttreatment blood loss was reduced in the ARC group, compared with the GAU group ($p < 0.01$), both on a body weight basis and overall (Figs. 1 and 2). No other differences were noted. No effect of treatment on fluid use was observed (Fig. 3). The percentage of animals in which hemostasis was attained was increased at both 3 ($p < 0.05$) and 4 ($p < 0.01$) minutes in the ARC group, compared with the GAU group, whereas no other differences were noted (Table 3). No differences in survival were observed. Dressing adherence strength scores were higher in the ARC group than in the GAU group ($p < 0.01$) and were reduced in the ALL group ($p < 0.01$). Mean adherence strength scores were 2.0 ± 0.2, 1.0 ± 0, 4.6 ± 0.1, 1.6 ± 0.2, 2.4 ± 0.2, and 1.8 ± 0.1, for the ACS, ALL, ARC, DAV, NYC, and GAU groups, respectively.

Examined across treatment groups, hemostasis was achieved in 17 animals and was not achieved in 62 animals. There were no differences between animals with and without hemostasis for any animal variable, or for pretreatment blood loss. However, differences were noted for some injury and outcome variables (Table 4). Pretreatment blood loss and survival were affected by number of vessels lacerated (Table 5). The number of vessels lacerated was positively correlated with pretreatment blood loss ($r = 0.30$; $p < 0.01$).

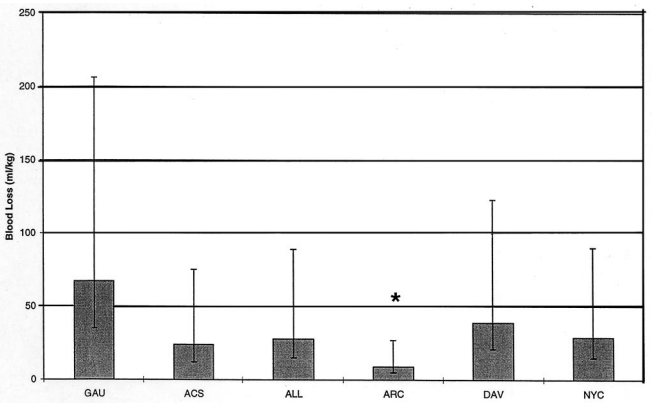

Fig. 2. *Effect of hemostatic dressing type on posttreatment blood loss per body weight. *Different from gauze control (p < 0.01). Means and 95% confidence intervals are shown.*

DISCUSSION

Injuries were equivalent across treatment groups, as demonstrated by similar numbers of vessels lacerated and similar pretreatment blood losses. These results are similar to those obtained in previous studies using this model.[32,34,36] This model involves extensive parenchymal and vascular damage. We have previously reported data from animals that were either untreated or were treated with perihepatic packing using this model.[32] These negative and "gold standard" con-

Table 3 Survival and Hemostasis in Animals Included in the Full Study

Group	Survival (%)	Hemostasis (%)			
		1 Min	2 Min	3 Min	4 Min
ACS	82	0	9	9	18
ALL	50	9	9	18	18
ARC	91	9	27	64[a]	73[b]
DAV	64	0	9	18	18
NYC	73	0	0	18	36
GAU	55	0	0	0	0

[a] Different from GAU ($p < 0.05$).
[b] Different from GAU ($p < 0.01$).

Table 4 Comparison of Animals with and with No Hemostasis

Variable	Hemostasis	No Hemostasis	*p* Value of Difference
No. of vessels lacerated	2.0 ± 0.2	3.4 ± 0.1	0.05
Survival (%)	100	47	0.01
Adherence strength score	3.4 ± 0.3	1.7 ± 0.1	0.01
Posttreatment blood loss (mL)	264 (155–450)	2,379 (1,864–3,037)	0.01
Posttreatment blood loss (mL/kg)	6.2 (3.7–10.6)	54.7 (42.9–69.6)	0.01

trols were not repeated in the present study. The vascular structures damaged in this animal model are approximately 1 cm in diameter. In human trauma, injury to major abdominal veins, such as the portal and hepatic veins, is associated with significant hemorrhage and mortality.[7] It is recognized that use of the present results to assess hemostatic potential of an HD for other severe venous applications is not without risk. However, we believe that the physical mechanisms involved in controlling large venous hemorrhage with a topically applied HD are sufficiently similar, regardless of the location of the damaged venous structure, to allow meaningful interpretation.

Across treatment groups, when hemostasis was attained, survival was 100% during the study period. Survival was reduced to less than 50% when hemostasis was not attained. The number of vessels lacerated was greater in animals that did not have hemostasis, suggesting that injuries with more vessels lacerated were hemostatically more severe and therefore more challenging for the hemostatic dressings. Injuries that involved more vessels also appeared to be more physiologically severe. Pretreatment blood loss was positively related to the number of vessels lacerated, whereas survival was negatively related to the number of vessels lacerated. These data indicate that although this hemorrhage model is severe both in terms of lethality and in terms of a hemostatic challenge to HDs, there is a range of severities related to the number of vessels lacerated. Dressings that used propyl gallate, aluminum sulfate, oxidized cellulose, microfibrillar collagen, fully acetylated poly-*N*-acetyl glucosamine, or thrombin as the principal active component failed to improve hemostasis, compared with the gauze control, in the present study.

The mechanism of propyl gallate as a procoagulant may be related to enhanced platelet procoagulant activity and annexin V binding.[46] Another derivative of gallic acid, bismuth subgallate, appears to activate factor XII.[47] Bismuth subgallate has been used as a component of a hemostatic paste for adenotonsillectomy, with equivocal hemostatic impact.[48] The efficacy of propyl gallate as part of a topical hemostatic has not been demonstrated.

Aluminum sulfate, commonly called alum, is classified pharmacologically as an astringent. Astringents are used to control capillary and small vessel bleeding for indications such as dental procedures,[49] bladder hemorrhage,[50] and various dermatologic procedures.[51]

Oxidized cellulose is an absorbable fiber derived from cellulose. The mechanism of action is believed to be based on its physical characteristics that allow it to provide a meshwork for coagulation. It has been used widely for the control of capillary bleeding in a variety of procedures.[51,52] In a canine arterial puncture model, oxidized cellulose with gauze was superior to gauze alone in controlling hemorrhage when applied with prior vascular control.[29] This material also controlled capillary splenic bleeding in a canine[20] and a porcine study.[21] In another study, oxidized cellulose failed to improve hemostasis compared with gauze or no treatment in a model of minor hepatic parenchymal hemorrhage in rats.[25]

Microfibrillar collagen is an absorbable form of collagen prepared from purified animal collagen fibrils. It may be used in the form of a fleece or as a powder.[51] Microfibrillar collagen has been used in a wide variety of procedures and tissues to control oozing and capillary hemorrhage.[51–54] Microfibrillar collagen was superior to absorbable gelatin sponge, oxidized cellulose, or no treatment in a dog model of splenic capillary bleeding.[20] It has also been effective during elective liver resection to control hepatic parenchymal bleeding.[53] The fleece form was effective when used for parenchymal and small venous and arterial hemorrhage of the kidney, liver, and spleen in swine.[20] Microcrystalline collagen was superior to manual pressure plus oxidized cellulose or manual pressure alone in a model of carotid arterial puncture (17 gauge) in dogs, when applied under conditions of vascular control.[29] However, microcrystalline collagen was no better than controls when used for bleeding after hemine-phrectomy in heparinized rats.[23] It was also ineffective when used to treat 10-mm longitudinal incisions in dog external iliac arteries with vascular control released 5 minutes after application.[16]

A fully acetylated poly-*N*-acetyl glucosamine sheet shortened time to hemostasis, compared with commercially available absorbable collagen and oxidized cellulose hemostatic products, in a model involving 3-mm-deep surface

Table 5 Effects of Number of Vessels Lacerated

No. of Vessels Lacerated	Percentage of Animals	Pretreatment Blood Loss	Survival	Hemostasis after 4 Min (%)
1	16	203 ± 44[a]	93[a]	36
2	36	306 ± 29[ab]	59[ab]	22
3	48	353 ± 25[b]	43[b]	12

[ab] Different superscripts within column differ ($p < 0.01$).

lacerations of the spleen and isolated splenic capsular stripping in swine.[21] Improved hemostatic activity compared with oxidized cellulose was also reported for this product when used for 3-mm-deep lacerations of the small intestine in human subjects.[21] In another study of splenic surface lacerations, a fully acetylated poly-*N*-acetyl glucosamine sheet reduced time to hemostasis compared with liquid fibrin glue in swine.[22] It also reduced time to hemostasis compared with absorbable collagen in a model of capillary splenic hemorrhage in heparinized swine.[22] An 80% deacylated poly-*N*-acetyl glucosamine membrane reduced bleeding after visceral and parietal peritoneal abrasion in a rabbit model.[54,55] No data have been published that document the successful use of a fully acetylated poly-*N*-acetyl glucosamine sheet for other than capillary bleeding.

Thrombin is a serine protease (MW, 36,000) that plays the pivotal role in coagulation by converting fibrinogen to fibrin.[56] Although thrombin has numerous physiologic functions,[57] it is used medically for its ability to form fibrin. Thrombin is used alone for the control of bleeding from cannulation sites, for the treatment of aneurysms, and for burn surgery.[58,59] Thrombin is combined with cryoprecipitate or other fibrinogen sources to form liquid fibrin glues[60] that are used for a wide variety of surgical applications.[61] Previous studies in which dry thrombin has been used without fibrinogen in the form of a dressing have demonstrated that thrombin-based dressings were ineffective in controlling hemorrhage from severe liver lacerations in normal[32] and coagulopathic swine[33] and after heminephrectomy in swine.[26] The results of the present study are consistent with the above findings.

The propyl gallate, aluminum sulfate, oxidized cellulose, microfibrillar collagen, fully acetylated poly-*N*-acetyl glucosamine, and thrombin treatments used in the present study failed to reduce blood loss or to enhance hemostasis. Previous reports of the efficacy of these approaches in the form of topical hemostats have been limited to capillary bleeding or to use in conjunction with vascular control or other measures. The lack of efficacy in the present study may have been because of the severe nature of the hemorrhage model used, which included rapidly flowing, large-volume hemorrhage from large vessels, parenchymal hemorrhage, and a bloody field without vascular control.

Numerous studies have documented the hemostatic efficacy of dressings that incorporate the fibrin glue components, fibrinogen and thrombin. A porcine collagen fleece soaked in liquid fibrin sealant was more effective than collagen fleece alone in treatment of liver injuries in rats, in terms of adhesive strength and 14-day survival.[28] Another study used a hemostat that consisted of fibrinogen (2.5–3.5 mg/cm^2), thrombin (0.5–1.0 U/cm^2), and aprotinin (1.6 μg/cm^2), all integrated into the surface of an equine collagen sponge. The fibrinogen-thrombin-collagen–based hemostat was effective when double wrapped over 10-mm longitudinal incisions in the external iliac arteries of dogs with 5 minutes of vascular control, whereas collagen alone was not effective.[16] The hemostatic dressing maintained hemostasis until arterial pressure was elevated to 260 mm Hg, when breakthrough bleeding occurred. In another study, a similar hemostatic dressing was effective for parenchymal bleeding of the liver and spleen in dogs and allowed breakthrough bleeding only when parenchymal pressures were elevated to 16.3 mm Hg.[24] Subsequent advancement of the fibrinogen-thrombin-collagen hemostatic dressing technology resulted in the development of the TachoComb dressing with a composition including an equine collagen sheet with human fibrinogen (4.3–6.7 mg/cm^2), bovine thrombin (1.5–2.5 U/cm^2), and aprotinin (0.055–0.087 Ph Eur U/cm^2), which has been used widely for surgical procedures in several countries.[54,62] A variation of this dressing without aprotinin has been used to stop bleeding in a heparinized rat heminephrectomy model that used vascular control.[23] More recently, another variation of the TachoComb dressing, with human thrombin substituted for bovine thrombin, was effective when used to seal suture lines after vascular anastomoses in human patients.[63] A different hemostatic dressing, using bovine collagen and bovine thrombin used in conjunction with autologous plasma, has been successfully used for diffuse hepatic parenchymal bleeding in human patients.[64]

Another approach has been to use higher fibrinogen and thrombin combinations plus calcium chloride and to use a nonhemostatic backing material for the construction of a dressing. An early prototype stopped arterial hemorrhage from freely bleeding 13-mm longitudinal incisions in the femoral arteries of pigs. The dressing was applied using 3.5 kg of pressure for a single 1-minute compression.[15] Variations of this dressing were later used to control arterial hemorrhage in swine[17] and arterial, venous, and diffuse bleeding from hind-limb gunshot wounds in goats, without vascular control.[41] In subsequent studies, a more refined dressing that included human fibrinogen (15 mg/cm^2), human thrombin (37.5 U/cm^2), and calcium chloride (117 μg/cm^2) freeze-dried onto a polygalactin mesh backing was used. In some studies, factor XIII (3.25 U/cm^2) was also added to the dressing. Using a liver injury model almost identical to that used in the present study, this dressing reduced blood loss compared with placebo controls in both normal and coagulopathic swine.[32–34] Others have demonstrated that the latter dressing reduced blood loss and operative time when used during prostatectomy in dogs[44] and partial nephrectomy in swine.[26]

In the present study, posttreatment blood loss was significantly reduced ($p < 0.01$) in the ARC group but not in the NYC group. There are several possible reasons for the differing results using two dressings with similar hemostatic constituents. It is possible that the factor XIII in the ARC dressing augmented function of the dressing. Factor XIII would be expected to stabilize the clot/seal formed by cross-linking fibrin strands. However, previous studies have demonstrated that the clot/seal formed using dressings similar to

the NYC dressing were stable, withstanding elevated arterial and parenchymal pressures.[16,24] Another possible explanation is the high concentration of calcium chloride present in the ARC dressing but not in the NYC dressing. This would be expected to hasten the reaction of thrombin with the fibrinogen in the dressing, potentially resulting in a more rapid clot/seal formation rate. A likely factor in the differing results noted is related to the different fibrinogen concentrations used in the two dressings. Fibrinogen concentration of fibrin sealants is positively related to "clot" strength.[60] In a study examining the effect of dressing fibrinogen concentration on blood loss after severe liver injury in swine, dressings that contained 0, 4, or 8 mg of human fibrinogen per square centimeter were not effective, whereas dressings containing 15 mg of human fibrinogen per square centimeter significantly reduced blood loss.[34] It is possible that several factors combined to result in different speeds of clot/seal formation and differing abilities to adhere in the presence of rapidly flowing blood. In each reported study using the fibrinogen-thrombin-collagen hemostatic dressing for the control of experimental hemorrhage, the dressing was applied with vascular control and allowed to form a seal for a period of minutes before vascular control was released. A slower clot formation rate is consistent with the lower fibrinogen and thrombin concentrations, and with the absence of calcium chloride in the NYC dressing.[60] The higher fibrinogen and thrombin concentrations and the inclusion of calcium chloride in the ARC dressing would be expected to result in a more rapid seal. The present results for the ARC group are consistent with previous studies in which the dressing was effective in the control of free-flowing arterial[15,27] and venous hemorrhage,[32–34] and hemorrhage associated with a severe renal stab wound.[37]

The requirements for an advanced HD to stop otherwise lethal hemorrhage was identified early in the development process. On the basis of this requirement, a physiologically and hemostatically severe model of large venous hemorrhage was selected. Use of this model allowed identification of a single effective dressing from among nine dressings with hemostatic properties. The ARC HD was the only HD that was effective in the present study. This dressing warrants further development. The model selection process used here resulted in a study that yielded data that are highly relevant and useful to the U.S. Army Advanced Hemostatic Dressing Development Program. We believe that this selection process may be applied to a broad range of research problems related to hemorrhage control.

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